

Fig. 1

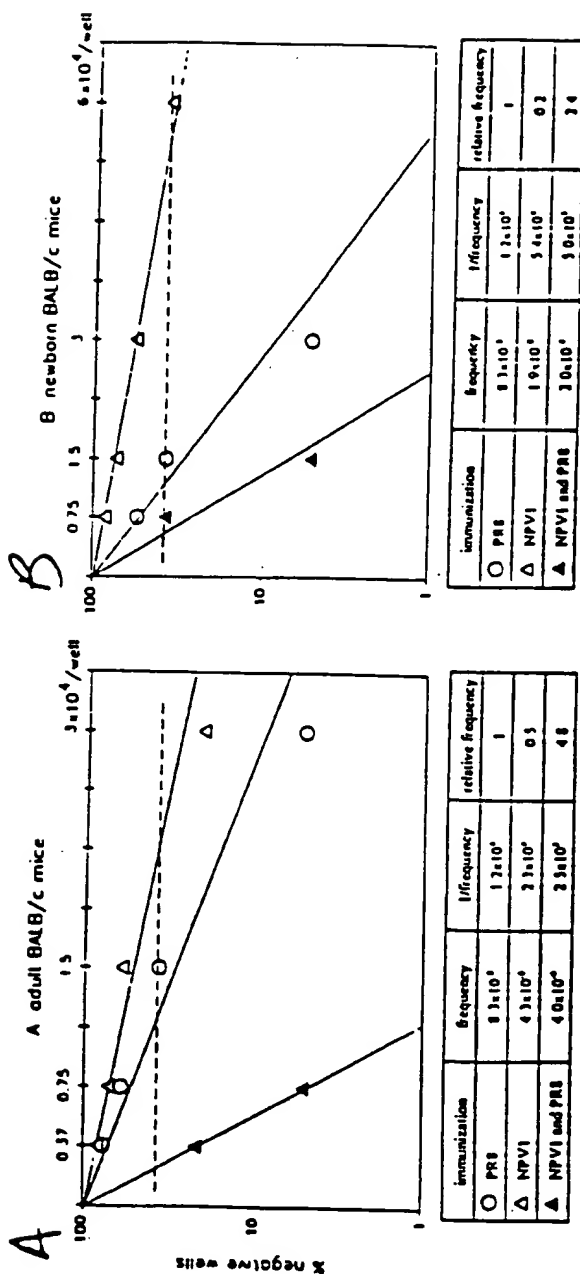
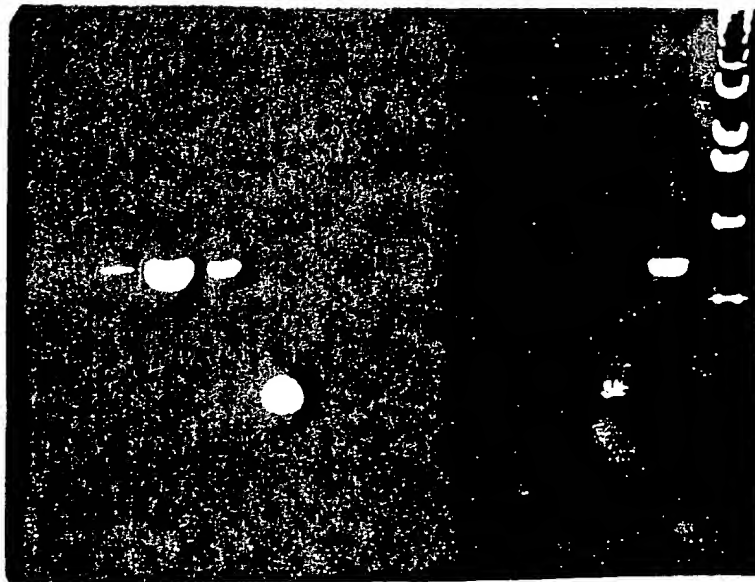


Fig. 2

1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13.



- 1- 4. Adult: right anterior tibial muscle.
- 5. Adult: left anterior tibial muscle.
- 6-10. Newborn: right gluteal muscle.
- 11. Newborn: left gluteal muscle.
- 12. NPV1 plasmid.
- 13. DNA ladder.

Fig. 3

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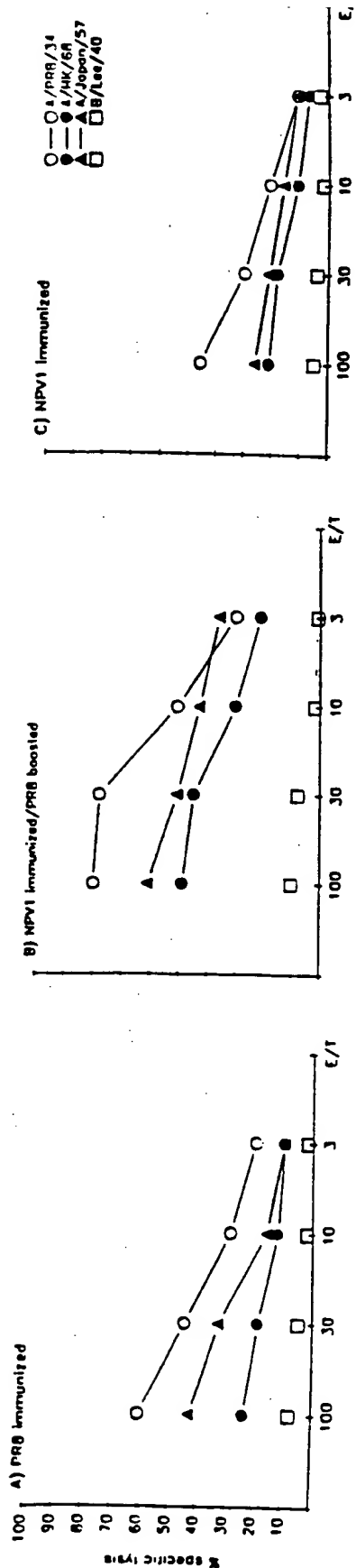


Fig 4

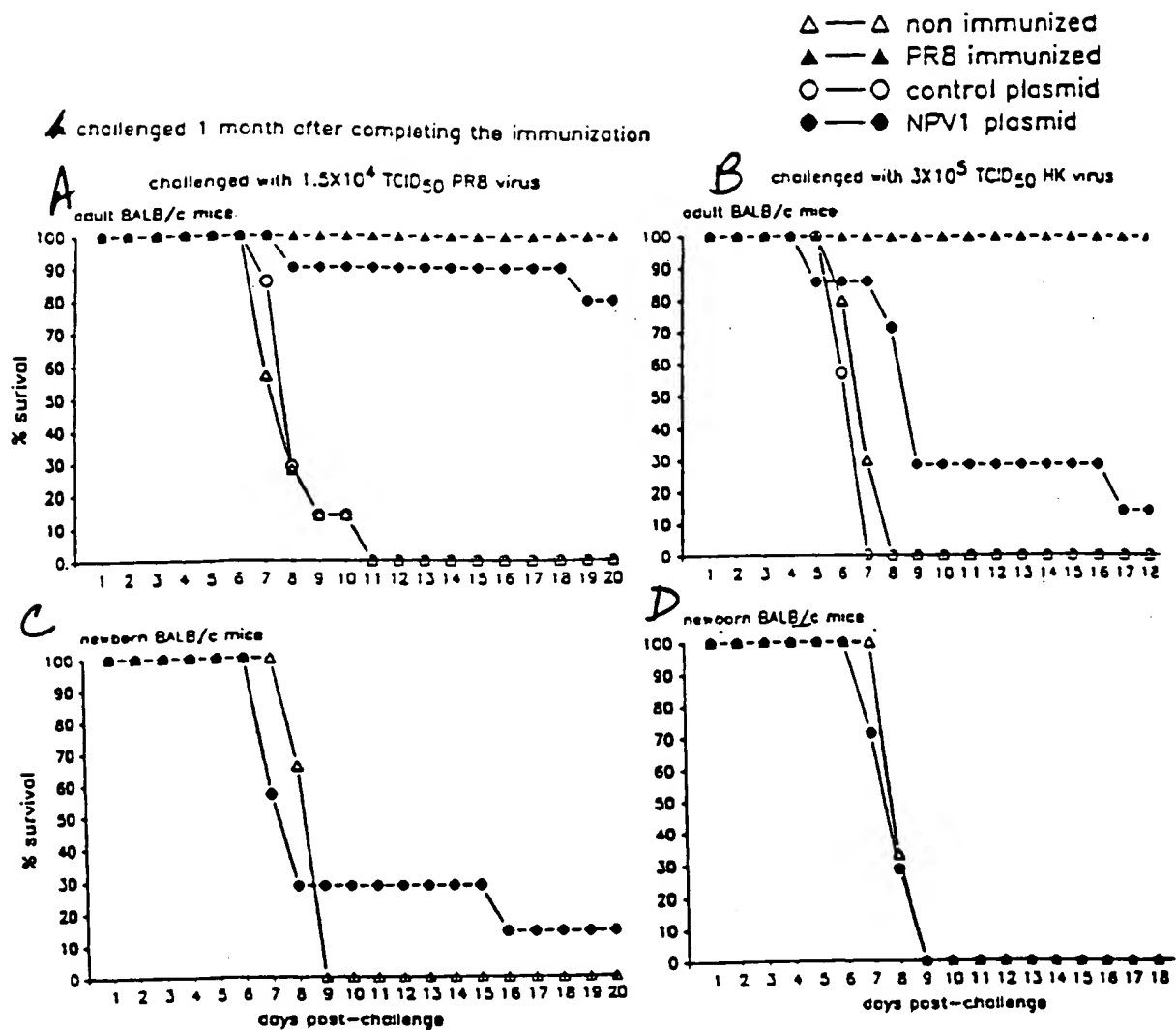
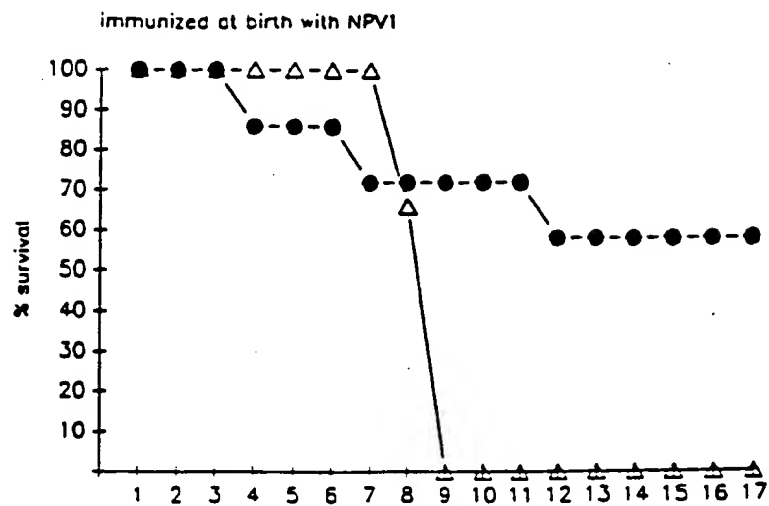


Fig. 5A

FO80E0"045F0B60

E

challenged 3 months after completing immunization, with 1.5×10^4 TCID₅₀ PR8 virus



F

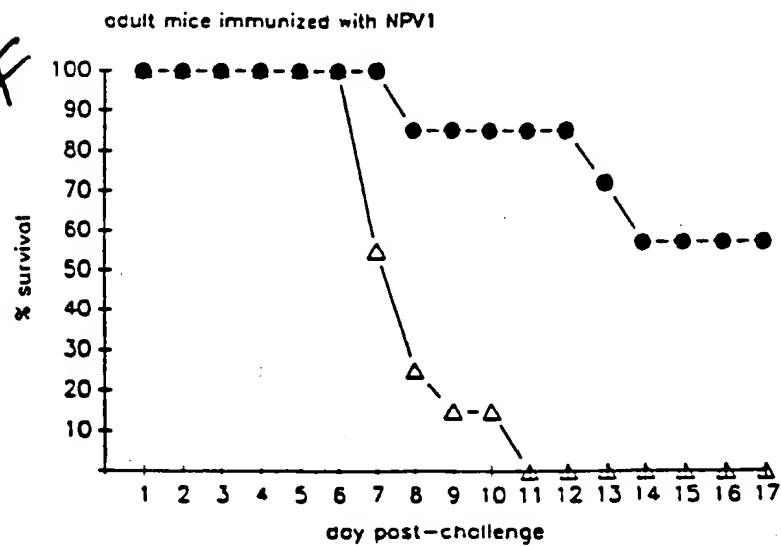


Fig. 5B

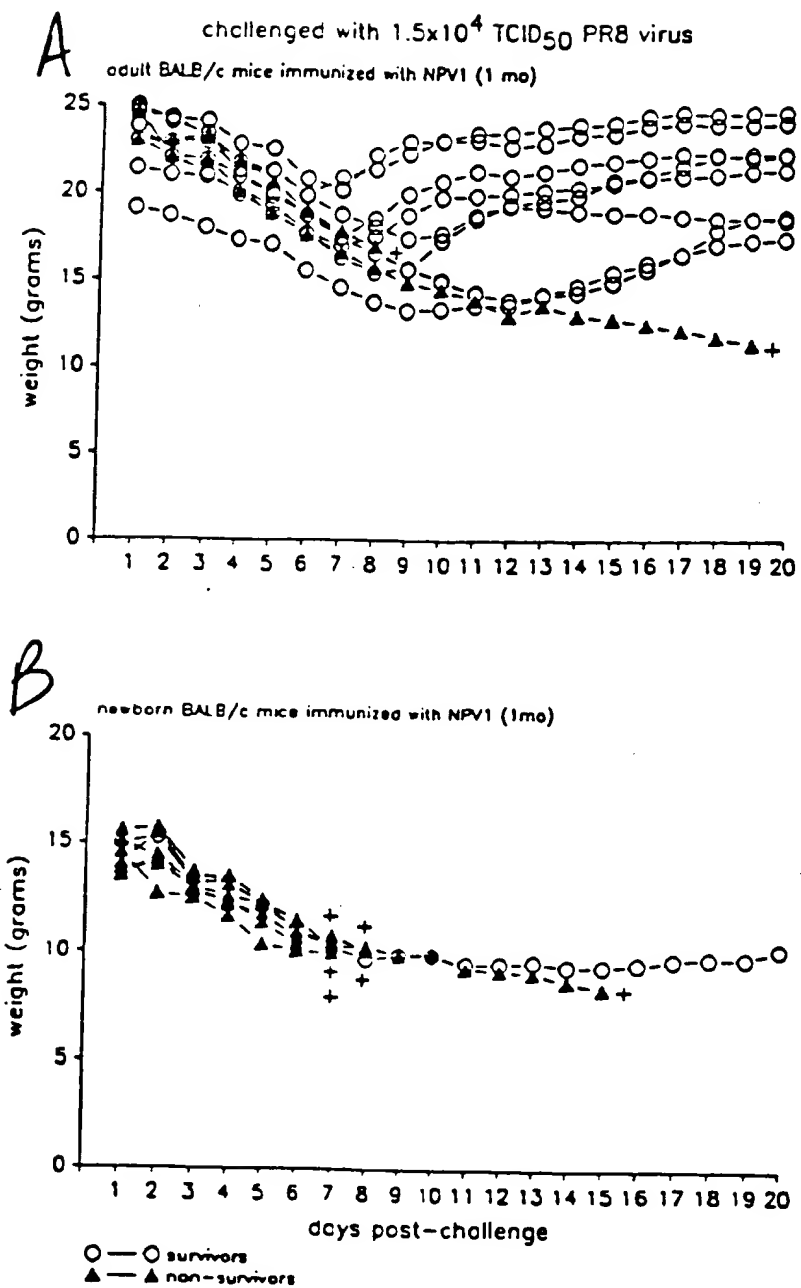


Fig. 6

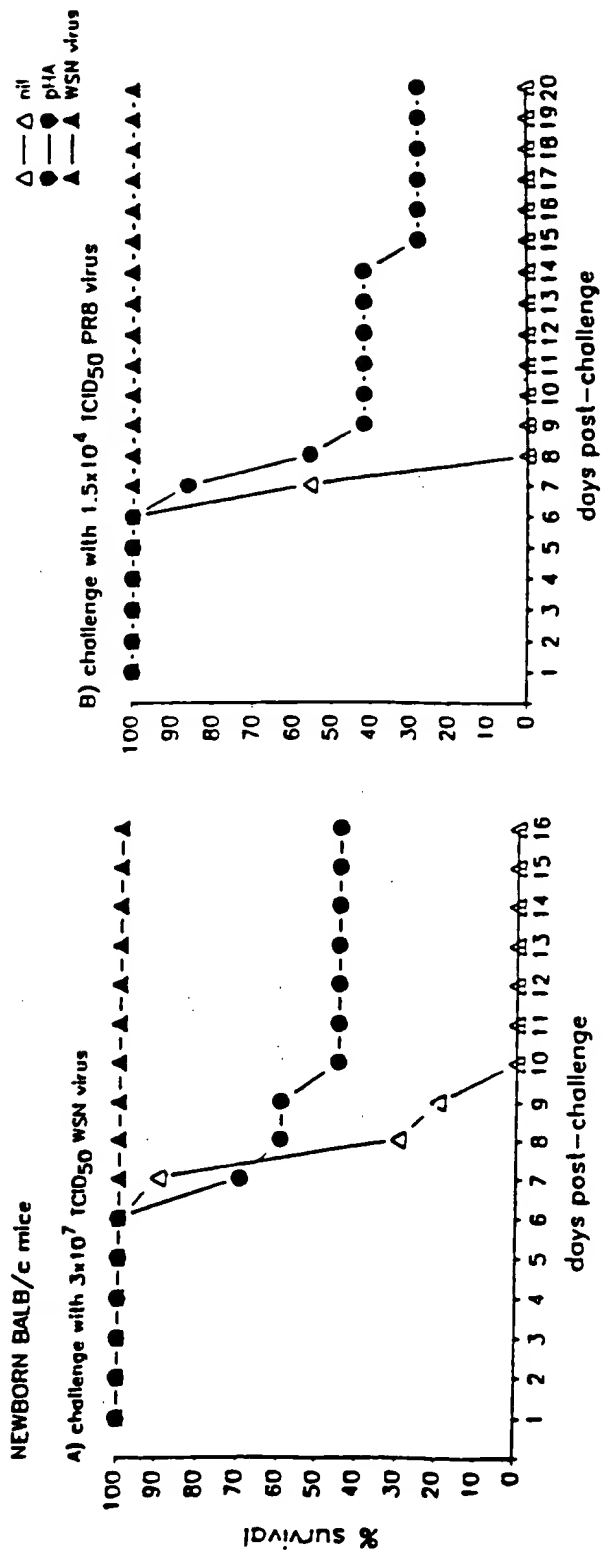


Fig. 7AB

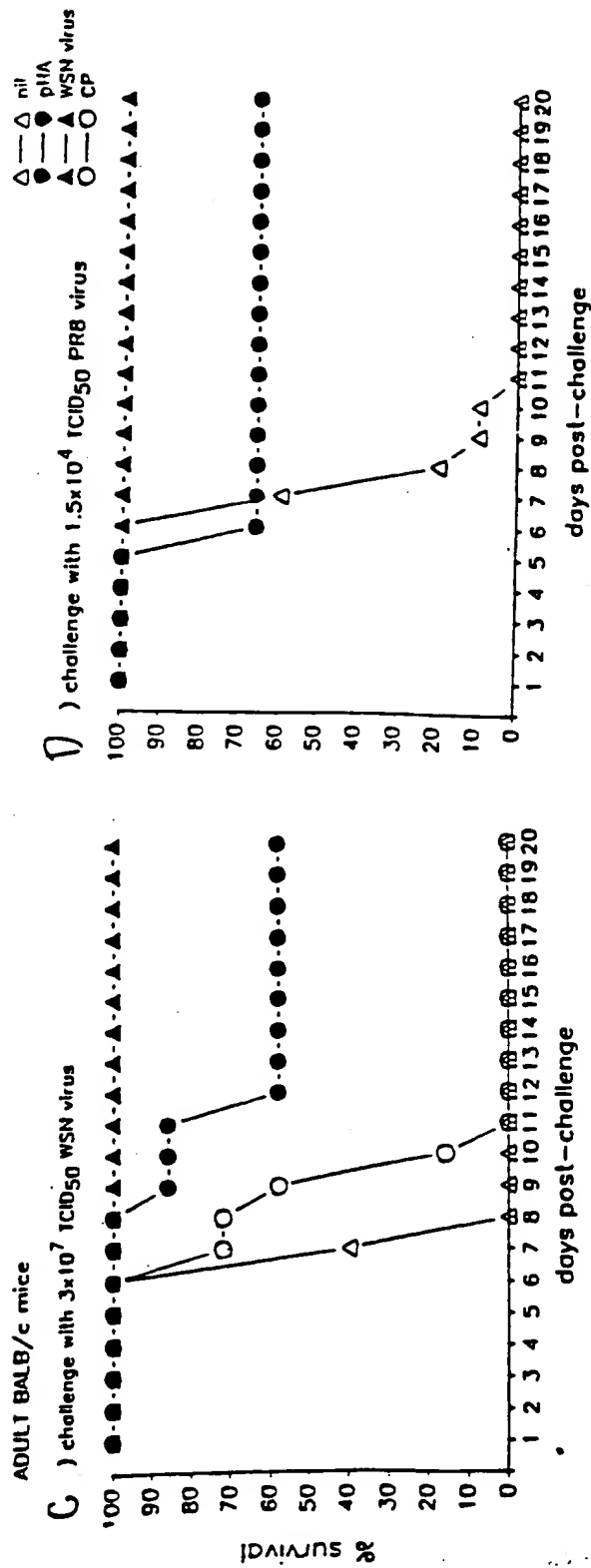
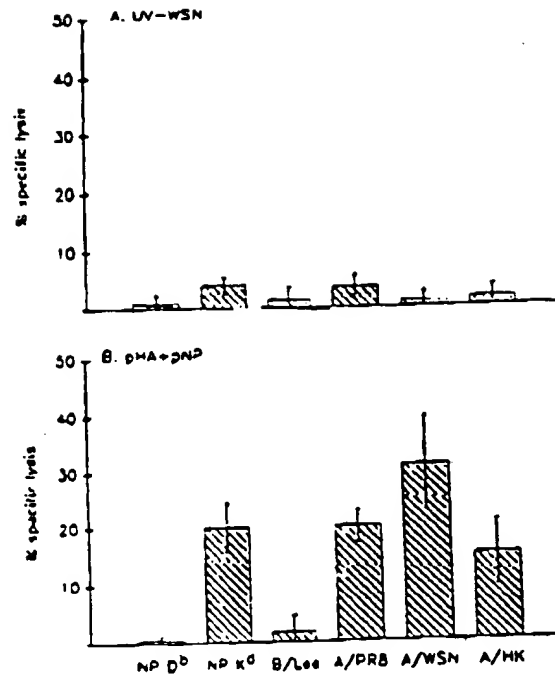
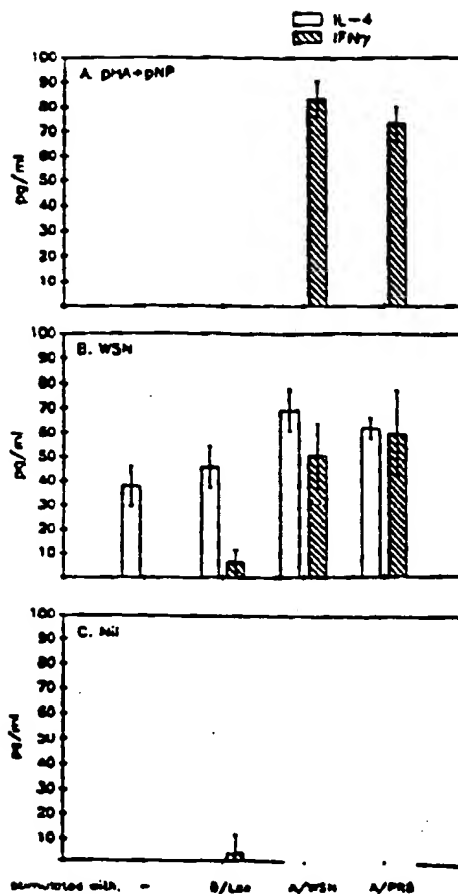


Fig. 7CD



CTL response of mice immunized as newborns with UV-attenuated WSN virus (A) or a combination of pHA and pNP plasmids (B). Splenocytes pooled from three mice in each group were *in vitro* stimulated with PR8 virus-infected APC and tested against P815 cells coated with NP peptides or infected with various Influenza viruses, at E/T ratio of 10:1. The results are expressed as means of % specific lysis \pm SD of triplicates.

Fig. 8



A

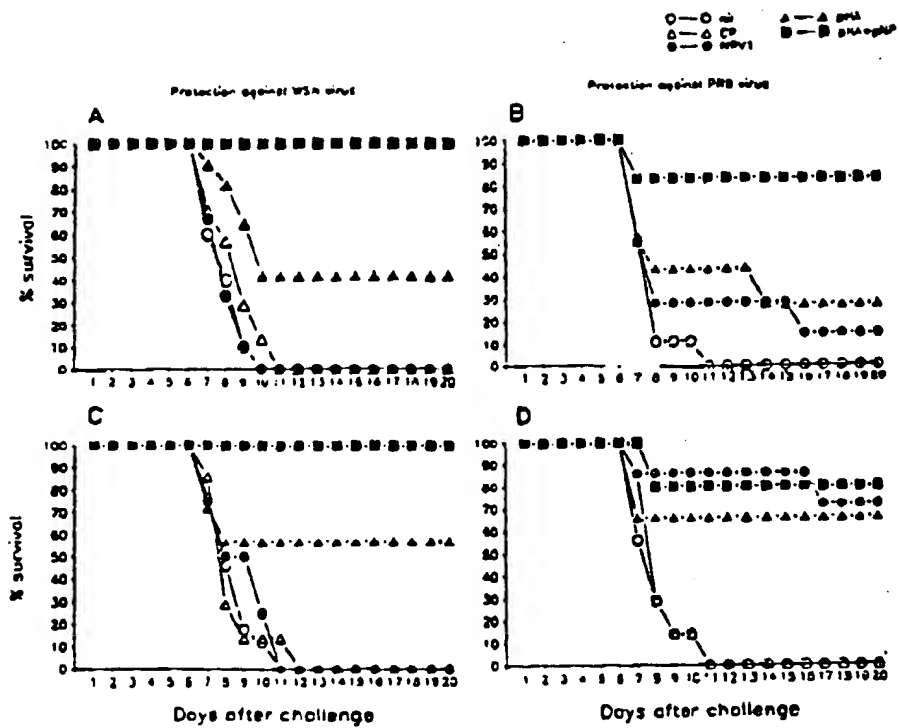
B

C

Cytokine secretion by CD4⁺ T cells from mice immunized as neonates with a combination of pHA and pNP plasmids (A), UV-attenuated WSN virus (B) or nil (C). Negatively selected CD4⁺ T cells were incubated four days in the presence of sucrose-purified UV-inactivated viruses (3μg/ml), APC and rIL-2 (6U/ml). The concentration of IFNγ and IL-4 was estimated by ELISA and the results were expressed as means of duplicates ± SD (pg/ml).

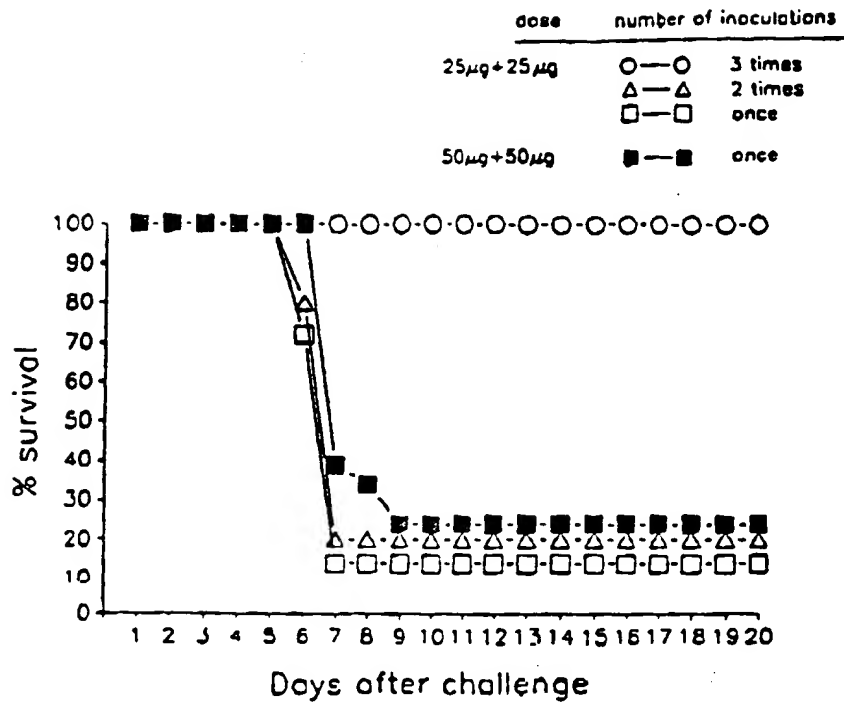
Fig. 9

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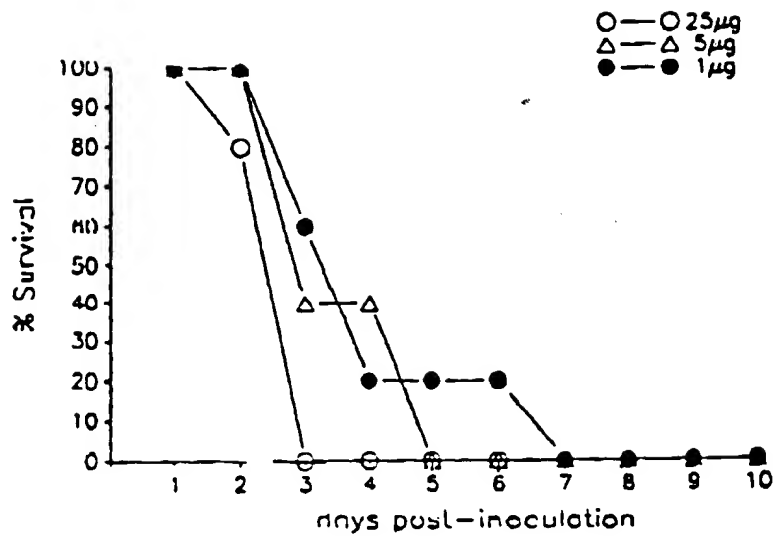
Protection against lethal challenge with WSN (A,C) or PR8 (B,D) virus of mice immunized as newborns (A,B) or adults (C,D) with a combination of pHA and pNP plasmids. As controls, we used naive mice, mice inoculated with a control plasmid (pRC/CMV) and mice immunized with pHA or pNP, separately. The mice were challenged with lethal doses of virus at four weeks following the completion of immunization.

Fig. 10



Dependence of the protection on the number of inoculations. The newborn mice were inoculated at day 1, 1 and 3, or 1, 3 and 6 with a mixture of pHA and pNP plasmids. At four weeks after the completion of immunization, the mice were challenged with a lethal dose of WSN virus.

Fig. 11

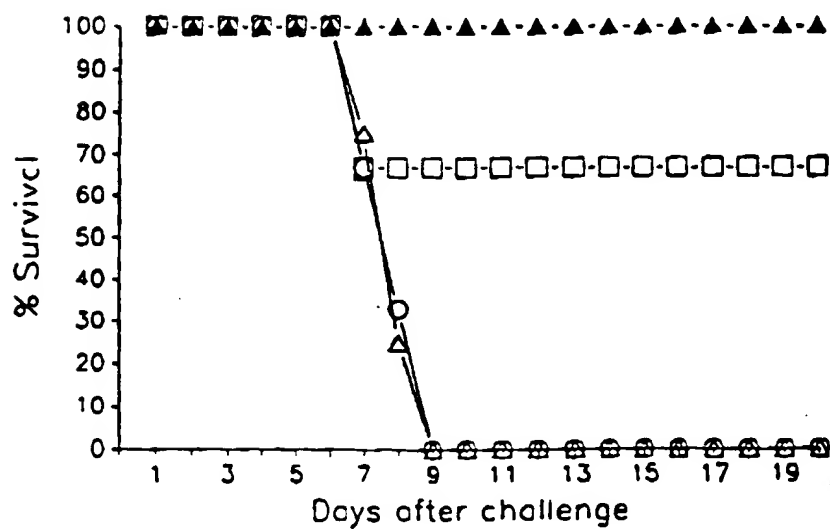


Lethality of live WSN virus following neonatal inoculation of mice. Various doses of live WSN virus were injected in the gluteal muscle of 1 day-old BALB/c mice. The survival of the neonates was followed for one week after the injection.

Fig. 12

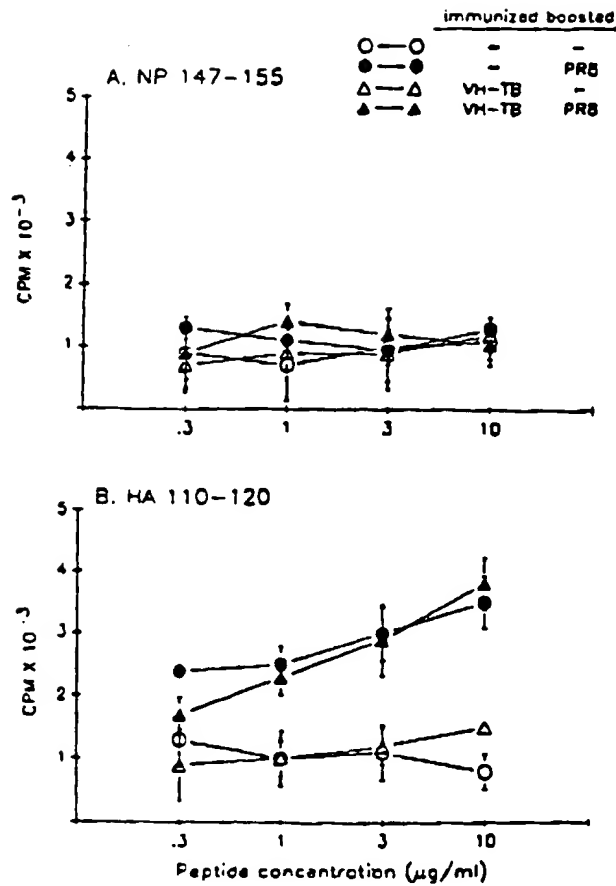
Group Immunized

| | | |
|---|----------|--------------------|
| ○ | neonates | 5 μ g UV-WSN |
| △ | neonates | 10 μ g UV-WSN |
| □ | adults | 5 μ g UV-WSN |
| ▲ | adults | 5 μ g live WSN |



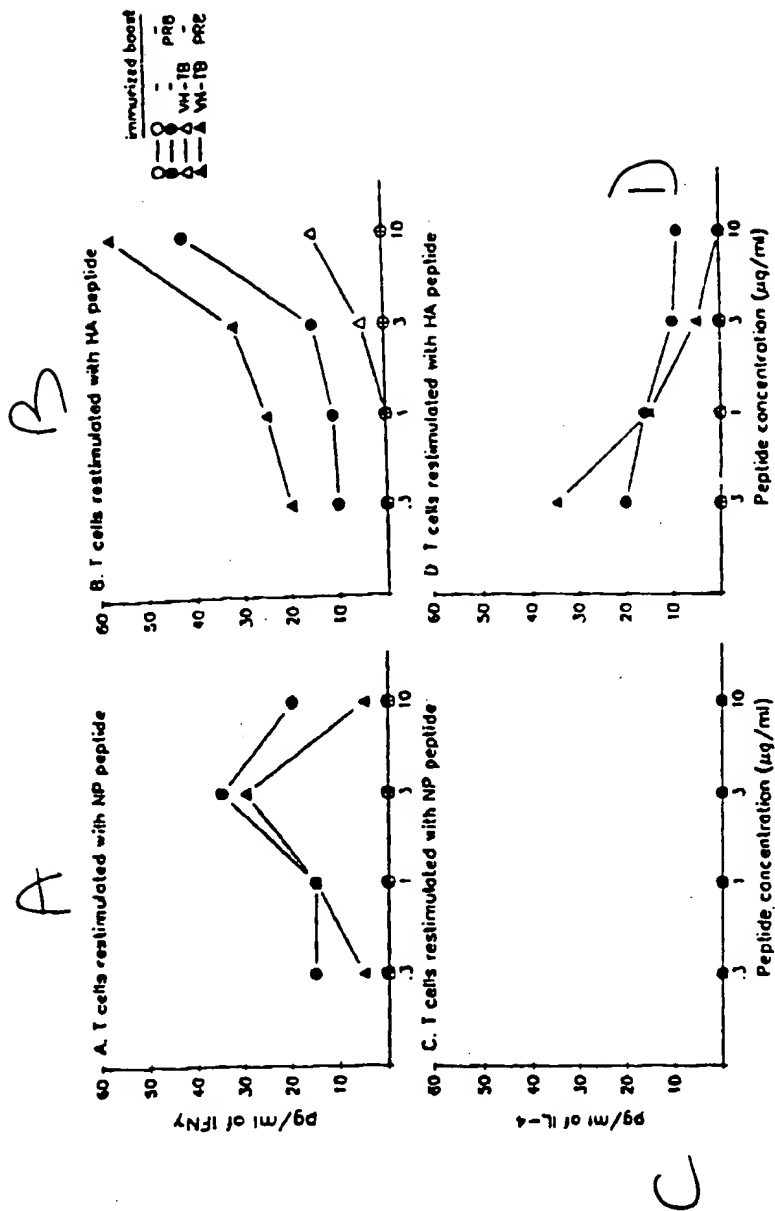
Protection against the homologous challenge of mice immunized as adults or newborns with WSN virus. Mice were inoculated i.m. with UV-inactivated or live-WSN virus. In the case of adult mice and challenged four weeks later with a lethal dose of WSN virus.

Fig. 13



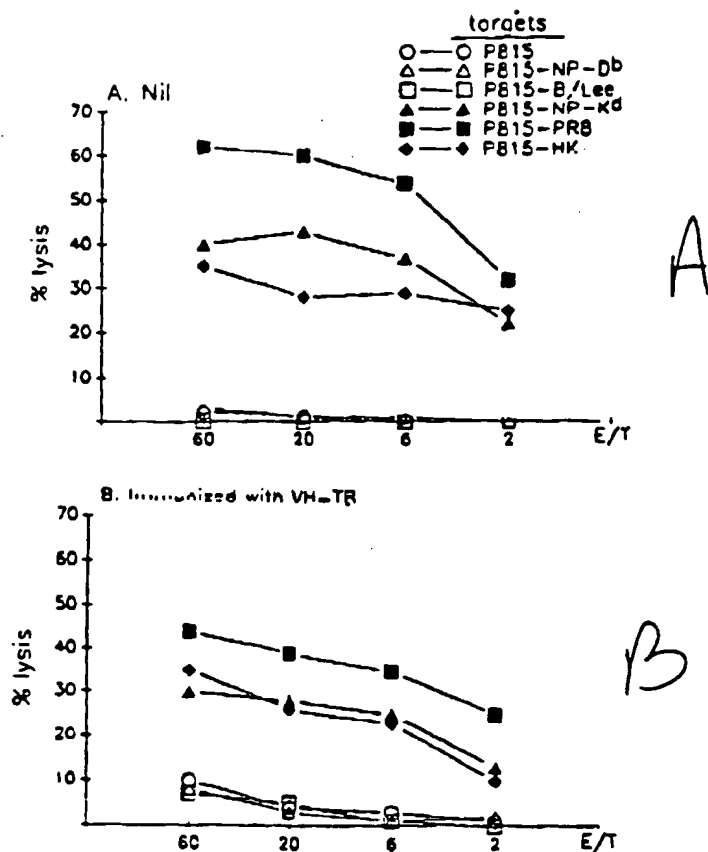
Proliferation of the CD4⁺ T cells from mice immunized as newborns with VH-TB plasmid. Negatively selected CD4⁺ T cells from mice immunized with VH-TB as neonates, were incubated with APC in the presence of various concentrations of NP 147-155 (A) or HA 110-120 (B) synthetic peptides. ³H-Thymidine was added after 72 hours and the radioactivity incorporated was measured after other 14 hours. The results are expressed as means of triplicates ± SD of proliferation indexes. Part of the mice immunized with VH-TB were boosted with PR8 virus. As controls, we used naive age-matched mice and mice immunized with live PR8 virus one week previous to the sacrifice.

Fig. 14



Cytokine production of the T cells from mice immunized as newborns with VH-TB plasmid. Nylon wool-purified T cells from spleens of mice immunized as neonates with VH-TB were incubated with various concentrations of NP 147-155 (A,C) or HA 110-120 (B,D) synthetic peptides in the presence of APC and 6U/ml rIL-2. IFN-γ (A,B) and IL-4 (C,D) were measured three days later by ELISA and the results were expressed as means of duplicates (pg/ml). SE was less than 25% of the mean, in each case. As controls, we used naive mice and mice immunized with PR8 virus one week previous to the sacrifice. Part of the mice immunized with VH-TB were boosted with PR8 virus one week previous to the study.

FIG. 15



The CTL response to PR8 virus of mice immunized as neonates with VH-TB plasmid. Mice immunized with VH-TB as newborns were boosted three weeks later with live PR8 virus. The splenocytes from three mice in each group (injected only with PR8 virus - (A) and immunized with VH-TB and boosted with PR8 virus - (B)) were harvested and pooled one week later and in vitro stimulated with PR8 infected APC. The cytotoxicity was measured against PB15 target cells infected with various strains of Influenza or coated with NP synthetic peptides. The results are expressed as means of % specific lysis of duplicates.

Fig. 16

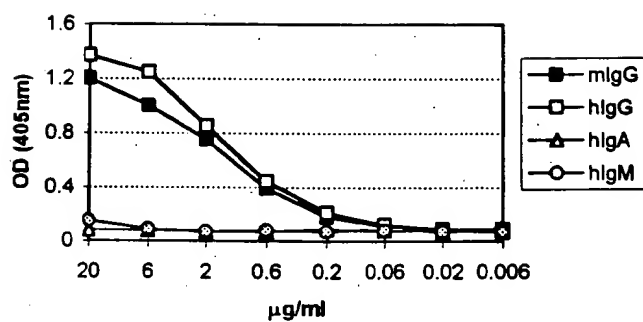


Figure 17

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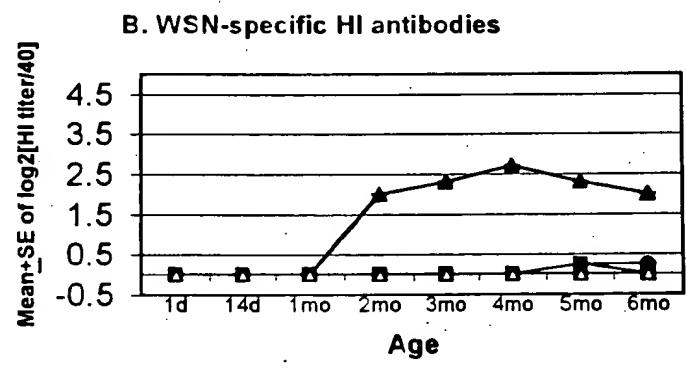
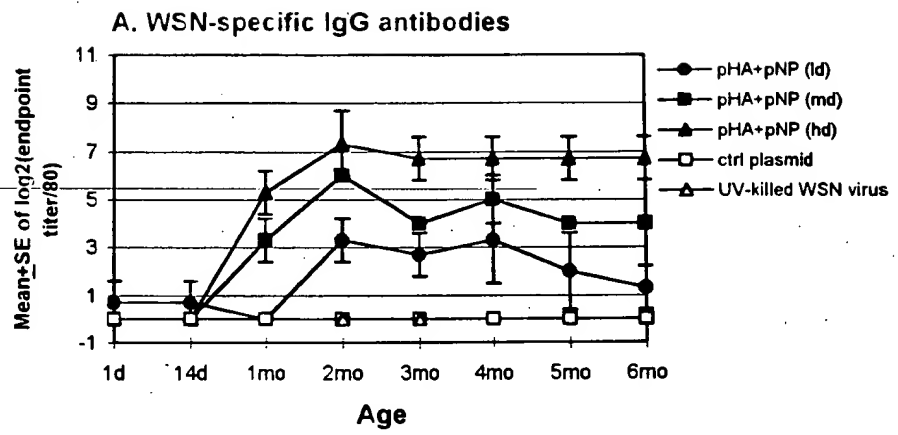
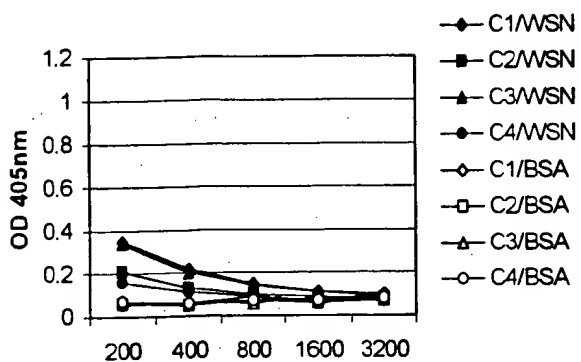
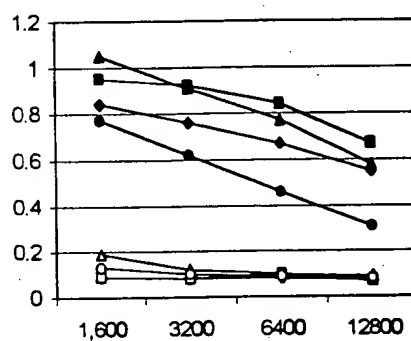


Figure 1B

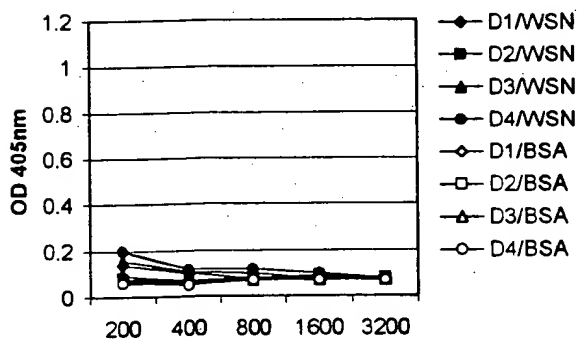
A. WSN-specific IgG before challenge
(pHA+pNP)



C. WSN-specific IgG after challenge
(pHA+pNP)



B. WSN-specific IgG before challenge
(Control Plasmid)



D. WSN-specific IgG after challenge
(Control Plasmid)*

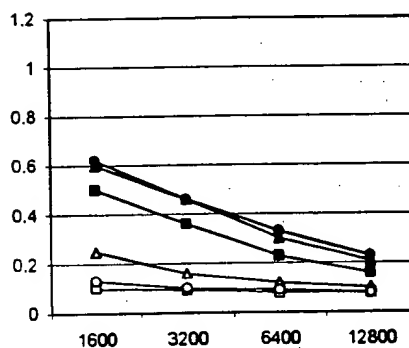


Figure 19

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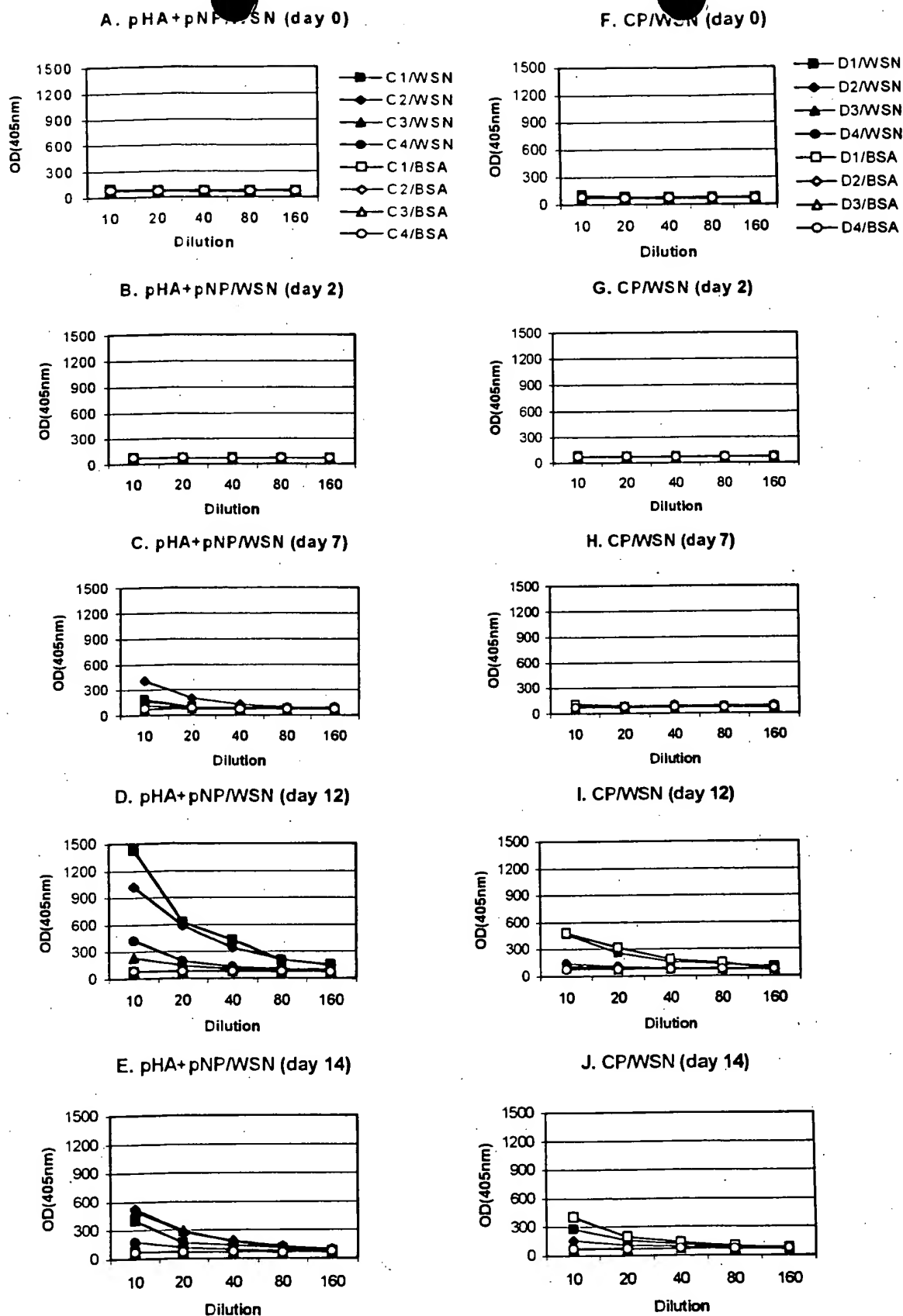


Figure 20

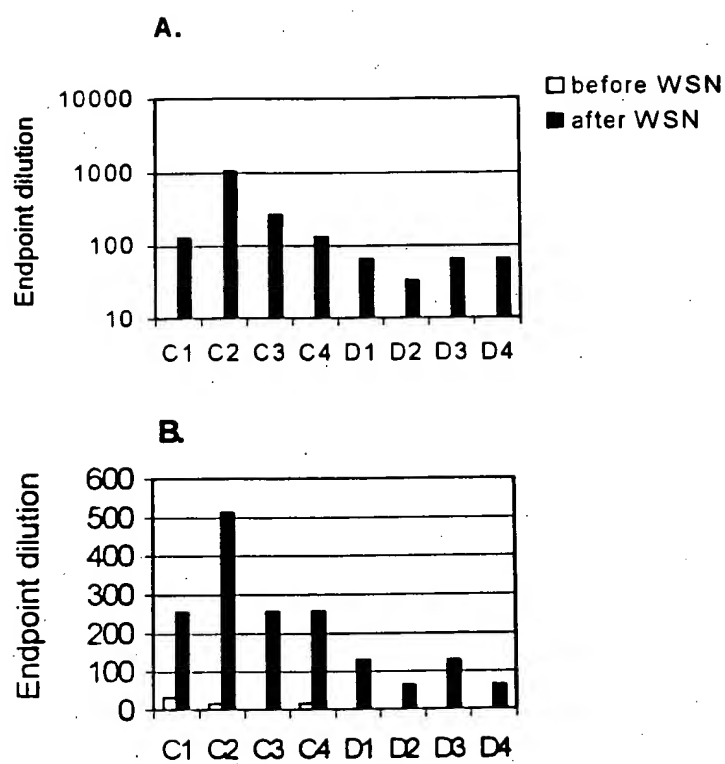


Figure 21